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APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
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08/746,635 11/13/96 MURTHY

V 96700/341  
EXAMINER

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HM31/0819

ART UNIT 3116, C PAPER NUMBER 18

DATE MAILED:

08/19/98

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

### OFFICE ACTION SUMMARY

- Responsive to communication(s) filed on 5/5/98, 11/24/97, 7/16/97
- This action is FINAL.
- Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

#### Disposition of Claims

- Claim(s) 1-4, 8-10, 19 is/are pending in the application.  
Of the above, claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- Claim(s) \_\_\_\_\_ is/are allowed.
- Claim(s) 1-4, 8-10, 19 is/are rejected.
- Claim(s) \_\_\_\_\_ is/are objected to.
- Claim(s) \_\_\_\_\_ are subject to restriction or election requirement.

#### Application Papers

- See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.
- The proposed drawing correction, filed on \_\_\_\_\_ is  approved  disapproved.
- The specification is objected to by the Examiner.
- The oath or declaration is objected to by the Examiner.

#### Priority under 35 U.S.C. § 119

- Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- All  Some\*  None of the CERTIFIED copies of the priority documents have been
- received.
- received in Application No. (Series Code/Serial Number) \_\_\_\_\_
- received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\*Certified copies not received: \_\_\_\_\_

- Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e).

#### Attachment(s)

- Notice of Reference Cited, PTO-892
- Information Disclosure Statement(s), PTO-1449, Paper No(s). \_\_\_\_\_
- Interview Summary, PTO-413 Dated 10/28/97 (paper no 13)
- Notice of Draftsperson's Patent Drawing Review, PTO-948
- Notice of Informal Patent Application, PTO-152

-SEE OFFICE ACTION ON THE FOLLOWING PAGES-

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***CONTINUED PROSECUTION APPLICATION ESTABLISHED***

The request filed on May 15, 1998 (paper no. 17) for a Continued Prosecution Application (CPA) under 37 CFR 1.53(d) based on parent Application No. 08/746,635 is acceptable and a CPA has been established. An action on the CPA follows.

***AMENDMENT ENTRY; CLAIM STATUS***

The Notice of Appeal filed November 24, 1997 (paper no. 14) is acknowledged and has been entered. Consequently, the amendment filed July 16, 1997 under 37 CFR 1.116 (paper no. 9) has been entered. Claims 13-15 have been cancelled. New claim 19 has been added. Claims 1-4, 8-10 and 19 are pending.

***INFORMALITIES***

The disclosure is objected to because of the following informalities: update the status of parent application USSN 08/421,079 on page 1 of the specification. Appropriate correction is required.

***DRAWINGS***

The drawings are objected to for reasons of record (see PTO-948 attached to paper no. 3). Correction is required.

The proposed drawing correction and/or the proposed substitute sheets of drawings, filed on July 16, 1997 (paper no. 10) have been approved by the examiner.

Formal correction of the noted defect can be deferred until the application is allowed by the examiner.

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***NON-ART BASED REJECTIONS***

Claims 1-4, 8-10 and 19 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 19 is confusing as to the difference between "level" (mass?) and "activity" (enzymatic activity?) of adenylate kinase. Claim 19 contains several antecedent basis problems. The following language or its equivalent is suggested for applicant's consideration.

19. (Amended) A method for determining [the level of] erythrocyte adenylate kinase enzymatic activity in a serum sample comprising the steps of:

(a) determining [the] total adenylate enzymatic activity in a first aliquot of the serum sample by mixing the [serum sample] first aliquot with [an] a first adenylate kinase-specific visualization agent which reacts with the total adenylate kinase causing a change in absorbance in the mixture, the change in then absorbance being indicative of the total adenylate kinase enzymatic activity;

(b) calculating [the percentage] percent of the erythrocyte adenylate kinase [to] in the total adenylate kinase in the serum sample by:

- (1) electrophoresing a second aliquot of the serum sample in a gel matrix so that the erythrocyte adenylate kinase migrates to a known location on the gel matrix;
- (2) contacting the gel matrix with [an] a second adenylate kinase-specific visualization reagent which reacts with the total adenylate kinase and causes emission of fluorescence upon exposure of the gel to ultraviolet light;
- (3) exposing the gel matrix to the ultraviolet light;

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- (4) [determining the level of the total adenylate kinase by] measuring [the] total [fluorescent] fluorescent light emitted from the gel matrix;
- (5) [determining the level of erythrocyte adenylate kinase by] measuring [the] fluorescent light emitted from the gel matrix at the known location of the erythrocyte adenylate kinase migration on the gel matrix; and
- (6) calculating the [percentage] percent of the erythrocyte adenylate kinase [to the total adenylate kinase] the measured fluorescent light of step (b)(5) by the measured total fluorescent light of step (b)(4); and
- (c) multiplying the [percentage] percent of the erythrocyte adenylate kinase by the total adenylate kinase enzymatic activity to give the [level of] erythrocyte adenylate kinase enzymatic activity in the serum sample.

#### ***ART BASED REJECTIONS***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Claim 1 is rejected under 35 U.S.C. 103(a) as being unpatentable over Olsson et al.

*(Journal of Applied Biochemistry, 5:437-445 (1983)).*

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Claims 2, 3 and 19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Olsson et al. (*Journal of Applied Biochemistry*, 5:437-445 (1983)) as applied to claim 1 above, and further in view of Tsuji et al. (Chemical Abstract 86:39099) or Friedrich et al. (*Biochemical Genetics*, 22 (5/6): 389-394 (1984)) and, if necessary, further in view of Buth et al. (Biological Abstract 71059076 (1981)).

Claims 4 and 8-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Olsson et al. (*Journal of Applied Biochemistry*, 5:437-445 (1983)) as applied to claim 1 above, and further in view of Matsuura et al. (*Journal of Biological Chemistry*, 264 (17): 10148-10155 (1989)).

The claimed invention is directed to (1) detection of hemolysis and/or conditions producing hemolysis by measuring serum adenylate kinase; and (2) determination of serum erythrocyte adenylate kinase activity.

Olsson et al. found that (i) adenylate kinase was concomitantly released with hemoglobin during cell aging; (ii) cell aging results in progressive lysis of erythrocytes; (iii) adenylate kinase was suitable for monitoring cell lysis due to its extreme storage stability; (iv) there was a high degree of correlation between the amount of accumulated hemoglobin and adenylate kinase; and, (v) while hemolysis was conventionally measured by measurement of extracellular hemoglobin, adenylate kinase activity measurement was also a sensitive and convenient way to follow hemolysis. An advantage of measuring adenylate kinase lies in studying the lysis of other cell types, e.g. platelets (see page 437, Table I, page 445). Olsson et al. determined adenylate kinase

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activity in plasma by measuring formation of ATP from ADP by the firefly luciferase reaction. DAPP, which is a specific inhibitor of erythrocyte adenylate kinase, confirmed the origin of the adenylate kinase in the plasma to be erythrocytic (page 442). Thus, Olsson et al. differs in detecting hemolysis by determining erythrocyte adenylate kinase activity in plasma rather than in serum. However, it would have been obvious to one of ordinary skill in the art to modify the method of Olsson et al. by determining erythrocyte adenylate kinase activity in serum rather than plasma because serum and plasma are conventional alternative sample types used in clinical analysis.

Olsson et al. also differs in failing to disclose alternative methods for determining erythrocyte adenylate kinase activity, e.g. including the use of gel electrophoresis and immunochemistry, which differentiate adenylate kinase activity of erythrocytic origin from adenylate kinase activity from other cells. Tsuji et al. measures erythrocyte adenylate kinase by agarose thin-layer gel electrophoresis with tetrazolium (i.e. formazan) visualization. Friedrich et al. describes electrophoretic separation and visualization of human erythrocyte adenylate kinase. Butch et al. use NAD-dependent glucose-6-phosphate dehydrogenase in adenylate kinase enzyme staining/detection procedures because it is significantly less expensive than utilizing NADP. Matsuura et al. describes immunoblot analysis of human erythrocyte adenylate kinase (AK1). Thus, it would have been further obvious and well within ordinary skill in the art to measure erythrocyte adenylate kinase by any known and conventional assay therefore, including electrophoretic separation and staining, such as with NAD-dependent glucose-6-phosphate

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dehydrogenase visualization technique, immunoassays, etc. as suggested by Tsuji et al., Friedrich et al., Buth et al. and/or Matsuura et al.

***Arguments and Rebuttals***

Applicant argues (1) Olsson et al. does not detect and distinguish serum erythrocyte adenylate kinase from adenylate kinase of other origins; (2) non-erythrocytes, i.e. platelets, release erythrocyte adenylate kinase, which non-erythrocyte adenylate kinase is also inhibited by DAPP; and (3) Tsuji et al., Friedrich et al., Buth et al. and Matsuura et al. determine total adenylate kinase NOT erythrocyte origin adenylate kinase.

In response, (1) it is respectfully submitted that Olsson et al. does distinguish that portion of total adenylate kinase which is due to erythrocyte adenylate kinase from adenylate kinase of other origins via addition of DAPP, which is a specific inhibitor of erythrocyte adenylate kinase (see page 442, ¶3). Indeed, the major thrust of Olsson et al. is drawn to erythrocyte adenylate kinase as shown by the constant attention drawn to erythrocyte adenylate kinase and the correlation between cell lysis, hemoglobin release and adenylate kinase release in red blood cells.

(2) Olsson et al. state explicitly on page 442, ¶3 that DAPP is a specific inhibitor of erythrocyte adenylate kinase, not platelet adenylate kinase. Moreover, Olsson et al. was studying the leakage of adenylate kinase from stored blood cells; and, confirming results by addition of *erythrocyte* adenylate kinase. Clearly, Olsson et al. teaches and/or suggests the critical correlation between hemolysis and erythrocyte adenylate kinase. (3) Insofar as Tjusi et al. teaches electrophoretic separation and visual definition/detection of the adenylate kinase

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isoenzymes, i.e. adenylate kinase from different cellular sources, it is respectfully submitted that Tjusi et al. suggest determination of erythrocyte adenylate kinase levels (see new claim 19 which also recites electrophoretic separation and determination of erythrocyte adenylate kinase). Fig. 2 in Friedrich et al. shows "Histochemical staining of human erythrocyte adenylate kinase". Buth et al. does not specifically address adenylate kinase isoenzymes. However, it is respectfully submitted that one of ordinary skill in the art would have found that suggestion implicit in Buth et al. by virtue of (a) the reference to "electrophoretic" staining procedures and (2) the references to enzymes commonly resolved by electrophoresis into their respective isoenzymes, e.g. creatine kinase. As to Matsuura et al., this reference teaches separation of adenylate kinase isoenzymes, e.g. AK1 (erythrocyte adenylate kinase), by column chromatography prior to determination of the enzymatic activity of each fraction eluted from the column.

The Court of Appeals for the Federal Circuit in *Interconnect Planning Corp.*, 227 USPQ 543 (Fed. Cir. 1985), stated that "Not only must the claimed invention as a whole be evaluated, but so also must the references as a whole, so that their teachings are applied in the context of their significance to a technician at the time." *Id.* at 551. A technician at the time would have appreciated the correlation between hemolysis and *erythrocyte* adenylate kinase taught/suggested by Olsson et al., and emphasized by the parallel between hemoglobin (a known indicator of hemolysis) and erythrocyte adenylate kinase taught/suggested by Olsson et al.; as well as the ability to differentially measure the *erythrocytic* isoenzyme of adenylate kinase, e.g. using ordinary and conventional means for differentially isoenzymes, such as immunoassay and electrophoretic

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separation, commonly used to differentially measure over isoenzymes, including creatine kinase and lactate dehydrogenase isoenzymes. Such basic techniques for differential isoenzyme measurement were well within ordinary skill in the art at the time of the instant invention. Therefore, these arguments are not convincing patentability for the above reasons and reasons of record.

#### ***MISCELLANEOUS REMARKS***

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.

Szasz et al. (*Clinical Chemistry*, 22(11):1806-1811 (1976)) discusses DAPP's inhibition of adenylate kinase isoenzymes.

Meiattini et al. (US 4,220,714) discusses inhibitors for adenylate kinase.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Carol A. Spiegel whose telephone number is (703) 308-3986.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, James Housel, can be reached on (703) 308-4027. The fax phone number for the organization where this application or proceeding is assigned is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Carol A. Spiegel  
August 7, 1998

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